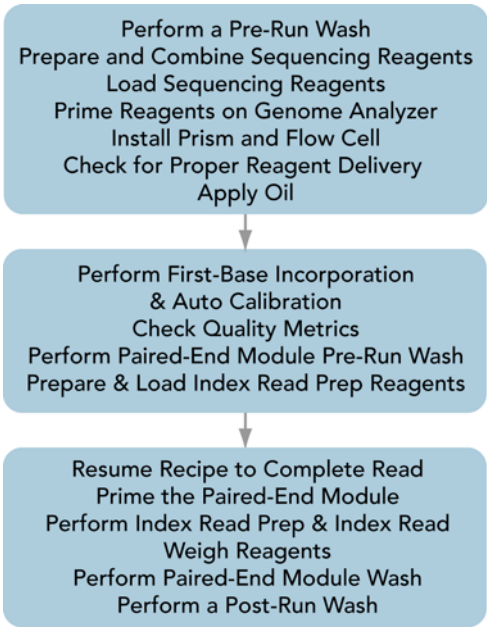
Multiplexed Single-Read Sequencing on the Genome Analyzer

Experienced User Card

This experienced user card explains how to perform a multiplexed single-read sequencing run on the Genome Analyzer using version 7 recipes and reagents provided in the Sequencing Kit v4. Multiplexing reagents are provided in the Multiplexing Sequencing Primers and PhiX Control Kit.

Workflow



Approximate Run Time

Number of Cycles and Run Type	Sequencing Kit v4 with v7 recipes Genome Analyzer *II*	Sequencing Kit v4 with v7 recipes Genome Analyzer *IIx*
101-cycle single-read	5 days	5.5 days
76-cycle single-read	3.5 days	4 days
51-cycle single-read	2.5 days	3 days
36-cycle single-read	2 days	2 days

Preparing for the Run

This section explains how to perform a pre-run wash, prepare and combine reagents for long runs, load and prime reagents, install the prism and flow cell, and apply immersion oil between the prism and flow cell.

Use reagents provided in the Sequencing Kit v4 and version 7 recipes.

Perform a Pre-Run Wash

[ ] **1.** Load the Genome Analyzer with a used flow cell.

[ ] **2.** Load 10 ml of PW1 onto positions 1, 3, and 6, and 40 ml of PW1 onto positions 2, 4, 5, and 7 on the Genome Analyzer.

[ ] **3.** Place at least 5 ml of laboratory grade water onto positions 19,21, and 22 on the Paired-End Module.

[ ] **4.** Bundle the Genome Analyzer waste tubing, keeping the ends even. Wrap the tubes with parafilm and place tube ends into a 50 ml bottle.

[ ] **5.** Select **File** | **Open Recipe** and open GA2-PEM_MP_PreWash_SR_v7.xml.

[ ] **6.** Click **Start**. Reagents are delivered 1 ml at a time. Run time is approximately 15 minutes.

[ ] **7.** Record the delivery volume on the lab tracking form.

Prepare Sequencing Reagents

Prepare reagents using the following table to determine the correct combination of 36-cycle and 18-cycle kits for the type of run and number of cycles you plan to perform.

Cycles Count	36-Cycle Kits	18-Cycle Kits
36-cycle multiplexed single-read run	1	1
51-cycle multiplexed single-read run	1	1
76-cycle multiplexed single-read run	2	
101-cycle multiplexed single-read run	3	

Thaw Reagents

[ ] **1.** Thaw the IMX, LFN, and SMX at room temperature or in a beaker containing deionized water. Leave the RDP in -15° to -25°C storage.

[ ] **2.** Thaw the CLM at room temperature or in a *separate* beaker containing deionized water. After handling the CLM container, discard your gloves and replace them with a new pair.

[ ] **3.** Record the lot numbers of each reagent on the lab tracking form.

[ ] **4.** Immediately after the reagents have thawed, place them on ice.

Prepare IMX

[ ] **1.** Transfer 1.76 ml (18-cycle kit) or 3.52 ml (36-cycle kit) of LFN into the IMX.

[ ] **2.** Remove the RDP tube from -15° to -25°C storage and briefly pulse centrifuge.

[ ] **3.** Transfer 110 µl (18-cycle kit) or 220 µl (36-cycle kit) of RDP into the IMX and LFN mix.

[ ] **4.** Cap the IMX tube tightly and invert five times to mix.

[ ] **5.** Centrifuge at 1,000 xg for one minute at 4°C, and then set aside on ice.

Prepare PR1, PR2, and PR3

[ ] **1.** Invert each bottle of PR1, PR2, and PR3 several times to mix.

Prepare SMX18

[ ] **1.** Invert the SMX18 tube several times to mix well.

[ ] **2.** Centrifuge at 1,000 xg for one minute at 4°C. Set aside on ice.

Prepare SMX36

[ ] **1.** Invert the bottle of SMX36 several times to mix well.

[ ] **2.** Pour the contents of the SMX container into one of the following containers:

- 175 ml Falcon bottle, if your Genome Analyzer is equipped with a large reagent chiller.
- SMX36 instrument tube provided in the kit, if your Genome Analyzer is not equipped with a large reagent chiller. Fill to the 50 ml mark.

[ ] **3.** Be sure the SMX is fully thawed and mixed. Cap the container and place the SMX on ice.

Prepare CLM

[ ] **1.** Invert the CLM tube several times to mix, and then centrifuge at 1,000 xg for one minute at 4°C.

[ ] **2.** Place the CLM in a *separate* ice bucket.

[ ] **3.** Discard your gloves and replace them with a new pair.

Combine Reagents

The following table shows how to combine reagents for long runs if you are using 175 ml bottles in positions 1, 3, and 6.

Position	Reagent	51-Cycle Run	76-Cycle Run	101-Cycle Run
1	IMX	Combine IMX18 and IMX36.	Combine one IMX18 and two IMX36.	Combine three IMX36.
3	SMX	Combine SMX18 and SMX36.	Combine one SMX18 and two SMX36.	Combine three SMX36.
4	PR1	Use PR1 from one kit.	Use PR1 from one kit.	Combine PR1 from two kits; fill to the neck of the bottle.
5	PR2	Use PR2 from one kit.	Use PR2 from one kit.	Combine PR2 from two kits; fill to the neck of the bottle.
6	CLM	Combine CLM18 and CLM36.	Combine one CLM18 and two CLM36.	Combine three CLM36.
7	PR3	Combine PR3 from both kits.	Combine two PR3.	Combine two PR3.

The following table shows how to combine reagents for long runs if you are using 50 ml tubes in positions 1, 3, and 6.

Position	Reagent	51-Cycle Run	76-Cycle Run	101-Cycle Run
1	IMX	Combine IMX18 and IMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two IMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two IMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.
3	SMX	Combine SMX18 and SMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two SMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two SMX36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.
4	PR1	Use PR1 from one kit.	Use PR1 from one kit.	Combine two PR1; fill to the neck of the 125 ml bottle.
5	PR2	Use PR2 from one kit.	Use PR2 from one kit.	Combine two PR2; fill to the neck of the 125 ml bottle.
6	CLM	Combine CLM18 and CLM36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two CLM36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.	Combine two CLM36 into a foil-wrapped 125 ml Nalgene bottle. Decant 50 ml into an amber tube. Store the remainder at 2° to 8°C.
7	PR3	Combine two PR3 into a 125 ml Nalgene bottle.	Combine two PR3 into a 125 ml Nalgene bottle.	Combine two PR3 into a 125 ml Nalgene bottle.

Load Sequencing Reagents onto the Genome Analyzer

[ ] 1. Record the weight of each reagent on the lab tracking form.

[ ] 2. Invert all reagent tubes several times.

[ ] 3. Load the prepared reagents onto the appropriate positions on the Genome Analyzer. Load the CLM last to avoid cross-contamination.

- Position 1—IMX
- Position 2—PW1
- Position 3—SMX
- Position 4—PR1
- Position 5—PR2
- Position 6—CLM
- Position 7—PR3

Prime Reagents

[ ] 1. Bundle all waste tubes so that the ends are even with each other, and wrap them with parafilm. Place the bundled tube ends into a 15 ml or a 50 ml conical tube.

[ ] 2. Select **File | Open Recipe** and open GA2_Prime_v7.xml, and then click **Start**.

[ ] 3. Collect the waste from the Genome Analyzer and ensure that the volume is 6.4 ml.

Unload Used Flow Cell and Prism

[ ] 1. Click **Load Flow Cell.**

[ ] 2. In the Pump area, make sure the following values are set to prevent siphoning of reagents.

Command: Pump
To: Flow cell
Solution: 8
Volume: 0
Aspiration Rate: 250
Dispense Rate: 2,500

[ ] **3.** Select **Instrument** | **Unlock Door** to release the door to the imaging compartment. Raise the door.

[ ] **4.** With the cursor in the **Dispense Rate** box, press **Enter**.

[ ] **5.** Raise the manifolds and slide the flow cell to the left to clear the manifolds.

[ ] **6.** Raise the beam dump.

[ ] **7.** Slide the metal prism base to the left to remove the prism.

Clean the Prism

[ ] **1.** Wearing new powder-free latex gloves, wipe down any spilled oil from the mounting rails, manifolds, Peltier heater, and beam dump.

[ ] **2.** Gently wash the prism with a stream of ethanol or methanol, and wipe the metal prism base with a lens cleaning tissue.

[ ] **3.** Using a lens cleaning tissue wet with ethanol or methanol, wipe off the surface of the prism in a single sweeping motion until it is clean.

Install the Prism

[ ] **1.** Ensure the surface of the prism holder is clean and free of oil.

[ ] **2.** Lift the beam dump, slide in the prism assembly, and then lower the beam dump until it locks into position.

Clean the Flow Cell

[ ] **1.** Wearing new gloves, hold the edges of the flow cell with two fingers. Ensure the inlet and outlet ports are facing *up*.

[ ] **2.** Wet a lens cleaning tissue with ethanol or methanol, and wipe off each side of the flow cell with a single sweeping motion.

Enter the Flow Cell ID

[ ] **1.** Click **Load Flowcell** on the manual control screen.

[ ] **2.** Click **Cancel** to retain the current flow cell ID, or enter the flow cell ID and click **OK**.

Load the Flow Cell

[ ] **1.** Using a lens cleaning tissue, gently apply pressure on the underside of the front manifold to absorb excess liquid.

[ ] **2.** Place the flow cell on top of the front and rear mounting rails with the inlet and outlet ports facing *up*. Press it gently against the stops on the right side.

[ ] **3.** Slide the flow cell toward the rear until you encounter the rear stop.

[ ] **4.** Test proper placement by applying gentle pressure toward the rear, then toward the right to ensure the flow cell is pressed against both stops.

[ ] **5.** While holding the flow cell against the stops with one hand, carefully rotate the manifold handle counterclockwise with the other hand to lower the manifolds into place.

Check for Proper Reagent Delivery

[ ] **1.** Wipe the interface of the manifold and the flow cell with a lens cleaning tissue.

[ ] **2.** Bundle all of the lines together with parafilm, making sure to keep the ends even, and then place the bundle into a 1.5 ml tube.

[ ] **3.** Pump 100 µl of Incorporation Buffer (solution 5) through the flow cell.

- [ ] **a.** Click the Manual Control/Setup tab.
- [ ] **b.** In the Pump area, set the values as follows:
  Command: Pump to Flow Cell
  To: Flow cell
  Solution: 5
  Volume: 100
  Aspiration Rate: 250
  Dispense Rate: 2,500
- [ ] **c.** With the cursor in the **Dispense Rate** box, press **Enter**.

[ ] **4.** Visually confirm that liquid is flowing properly through the flow cell.

[ ] **5.** Check for leaks where the flow cell touches the manifold using a lens cleaning tissue.

[ ] **6.** Measure the flow for each of the eight lanes three times and record the measured volumes on the lab tracking form. The expected volume is 800 µl.

Apply Oil

[ ] **1.** Aspirate 100 µl (Genome Analyzer *II*) or 150 µl (Genome Analyzer *IIx*) of oil into the pipette.

[ ] **2.** Place the pipette tip on the prism at the gap between the top surface of the prism and the front-left side of the flow cell, about 1 cm beyond the inlet manifold. Hold the pipette with two hands, using one hand to support and guide the tip.

[ ] **3.** Dispense the oil slowly from the left side; dispensing too fast will result in oil on the top of the flow cell.

[ ] **4.** Before the oil wicks to the right side of the flow cell, slide the pipette tip in small steps towards the rear, steadily dispensing more oil along the way.

[ ] **5.** Stop moving the pipette when the tip is about 1 cm short of the rear manifold.

[ ] **6.** Inspect the gap underneath the flow cell and ensure the following statements are true:

- There is a uniform layer of oil.
- No bubbles exist between the flow cell and prism.
- There is no oil on the top of the flow cell.
- The right surface of the prism is clean.

[ ] **7.** Use an ethanol wipe to clean the bottom surface of the Peltier heater.

[ ] **8.** Close the instrument door.

Performing the Run

This section describes the steps required to perform first-base incorporation and start a run on the Genome Analyzer.

Use a version 7 multiplexed single-read sequencing recipe with the following protocol. Only a version 7 recipe is compatible with Sequencing Kit v4.

Perform First-Base Incorporation

[ ] **1.** Select **File** | **Open Recipe** and open GA2_FirstBase_v7.xml.

[ ] **2.** Click **Start**. The recipe proceeds to first-base incorporation.

First-base incorporation chemistry takes approximately 20 minutes. Directly following first-base incorporation, the recipe performs auto calibration to find the best focus.

View Data in the Results Window

[ ] **1.** Click **View** | **Calibration Results** from the Data Collection toolbar.

[ ] **2.** Confirm the specifications are within range and click **Accept**. If necessary, move to another tile and repeat auto calibration.

View Data in Run Browser

[ ] **1.** View the First-Cycle Report to assess cluster counts, intensity values, and focus metrics.

[ ] **2.** Load the run log files to assess the quality of the data and decide whether to continue the run.

Perform the Run

If you are satisfied with the results of first-base incorporation, follow these instructions to perform the sequencing run.

[ ] **1.** If necessary, modify the recipe to perform the number of cycles required for your run using the recipe editor.

[ ] **2.** Select **File** | **Open Recipe** and open one of the following recipes:

- GA2-PEM_MP_101Cycle+7_SR_v7.xml (Paired-End Module)
- GA2-PEM2X_MP_101Cycle+7_SR_v7px.xml (Paired-End Module *IIx*)

[ ] **3.** Click **Start**. The recipe proceeds to a Paired-End Module pre-run wash.

Perform a Paired-End Module Pre-Run Wash

The Paired-End Module pre-run wash verifies correct operation of the Paired-End Module and is included in version 7 recipes.

[ ] **1.** Load water onto the Paired-End Module in positions 19, 21, and 22.

[ ] **2.** Click **OK.** Upon completion, measure the volume in each tube to confirm proper delivery. Click **OK** to proceed.

[ ] **3.**

Prepare Reagents for Index Read Prep

Multiplexing reagents are provided in the Multiplexing Sequencing Primers and PhiX Control Kit. You also need PW1 from the Sequencing Kit.

Thaw Reagents

[ ] Thaw reagents at room temperature or in a beaker containing deionized water. Do not thaw the Multiplexing Rd2 Seq Primer at this time.

Prepare 0.1 N NaOH

[ ] **1.** Invert the container of 2 N NaOH five times to mix the reagent, and then pulse centrifuge the reagent.

[ ] **2.** Transfer 1.9 ml of PW1 (provided in the Sequencing Kit) into a 15 ml Sarstedt conical tube and add 100 µl of 2 N NaOH.

[ ] **3.** Invert the tube five times to mix the reagent.

[ ] **4.** Label the conical tube of 0.1 N NaOH "Reagent #19."

[ ] **5.** Centrifuge at 1,000 rpm for one minute, and then set aside at room temperature.

Prepare Wash Buffer

[ ] **1.** Invert the container of Wash Buffer five times to mix the reagent, and then transfer 4 ml of Wash Buffer into a 15 ml Sarstedt conical tube.

[ ] **2.** Label the tube "Reagent #21." Set aside at room temperature.

Prepare Index Seq Primer

[ ] **1.** Transfer 1,990 µl of Hybridization Buffer into a 15 ml Sarstedt conical tube, add 10 µl of 100 µM Index Seq Primer, and then vortex to mix.

[ ] **2.** Label the tube "Reagent #22." Set aside at room temperature.

Load Index Read Prep Reagents onto the Paired-End Module

[ ] **1.** Record the weight of each reagent on the lab tracking form.

[ ] **2.** Load the reagents onto the Paired-End Module and connect the tubes to the corresponding port positions.

- Position 19—0.1 N NaOH
- Position 21—Wash Buffer
- Position 22—Index Seq Primer

Perform Read 1 and the Index Read

[ ] **1.** Click **OK** to resume the recipe.

Imaging of Read 1 begins. When Read 1 is complete, the recipe proceeds to priming of the Paired-End Module for Index Read Preparation.

[ ] **2.** If you are performing a run longer than 36 cycles and your Genome Analyzer is not equipped with a large reagent chiller, prepare to replace reagents during the run.

[ ] **3.** When priming of the Paired-End Module is complete, weigh each of the reagents to check the priming delivery. Record the weights on the lab tracking form.

[ ] **4.** When Index Read preparation is complete, weigh each of the reagents used on the Paired-End Module and record the weights on the lab tracking form.

[ ] **5.** When the run is complete, weigh each of the reagents on the Genome Analyzer and record the weights on the lab tracking form.

[ ] **6.** When prompted, replace all reagent tubes on the Paired-End Module with 15 ml conical tubes containing at least 10 ml of MilliQ water or laboratory grade water. Click **OK**.

Check Error Log

[ ] 1. Open the ErrorLog.txt located in <Run Folder>\Data.

[ ] 2. Check the entries.

Perform a Post-Run Wash

[ ] 1. Load 10 ml of PW1 in positions 1, 3, and 6, and 40 ml of PW1 onto positions 2, 4, 5, and 7 on the Genome Analyzer.

[ ] 2. Place at least 5 ml of laboratory grade water onto positions 19,21, and 22 on the Paired-End Module.

[ ] 3. Select **File** | **Open Recipe** and open GA2-PEM_MP_PostWash_SR_v7.xml and click **Start**.

[ ] 4. Click **Start**. Reagents are delivered 1 ml at a time. Run time is approximately 45 minutes.